Head and Neck Pathol (2008) 2:13–18
DOI 10.1007/s12105-007-0032-3

ORIGINAL RESEARCH

Estrogen and Progesterone Receptor and p53 Gene Expression in Adenoid Cystic Cancer

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Received: 26 June 2007 / Accepted: 26 September 2007 / Published online: 26 October 2007

Abstract *Objectives* The current study examined the role of estrogen receptors (ER), progesterone receptors (PR) and p53 expression in adenoid cystic carcinoma (ACC) to determine if simple expression or possible overexpression of these products might influence the development and natural course of this cancer. *Study Design* ER and PR status and p53 overexpression were retrospectively evaluated utilizing immunohistochemical evaluation of 47 ACC specimens. *Methods* Formalin-fixed paraffin-embedded tissues from 47 ACC specimens and 47 samples of normal salivary gland tissue were evaluated histochemically for the presence of ER, PR and p53. Immunoreactivity was scored using a 0 to +3 scale in which staining was either (0) negative, (+1) spotty, (+2) weakly positive, or (+3) strongly positive. *Results* ER was expressed in 8 of 47 tumors while PR was expressed in 4 of 47 tumors. p53 aberrations were demonstrated in 26 of 47 tumors. Tumors showed varying degrees of immunopositivity ranging from 0 to +3. *Conclusions* These studies suggest that p53 aberrations may be involved in ACC tumor progression and that ER and PR may play a role in ACC development.

Keywords Adenoid cystic carcinoma · p53 gene · Estrogen and progesterone receptors

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Introduction

Adenoid cystic carcinoma (ACC) is a rare and insidious malignant neoplasm that occurs most frequently in salivary glands. The tumor has a remarkable similarity to certain adenocarcinomas of the breast. In fact, some authors have reported that ACC is indistinguishable from ACC of the breast and vulva [1, 2]. No standard histologic parameters have proven to be reliable markers of local recurrence or distant metastases in cases of ACC. However, histologic sub-classification of this tumor into tubular, cribriform and solid subtypes can be predictive of biologic behavior, with the solid subtype being the most clinically aggressive of the three subclasses. Over the long-term, ACC has a remarkable ability to metastasize yet the short term and intermediate clinical biologic behavior of ACC is often difficult to predict.

The development of assessment markers would therefore seem to be of significant value in managing this disease. Although no assessment parameters have proven to be absolute predictors of stable or recurrent disease, there are numerous reports in the literature delineating a

possible association between salivary gland and breast cancer [3–6]. Dimery [6] analyzed estrogen receptors (ER) in normal salivary gland tissue and salivary gland carcinoma and found that eight of nine salivary gland tumors he evaluated in women had ER levels that would be considered hormone-dependent in breast carcinoma. Half of the cases reported were ACC.

Some studies also suggest that not only is estrogen expressed in breast ACC, but also that progesterone receptors (PR) may be expressed as well. ER and PR expression have been associated with a more favorable prognosis and increased survival in breast cancer [7–9]. In addition, an active estrogen-signaling pathway attenuated p53 apoptotic responses leading to tumor formation [10].

The current study uses immunohistochemical assays to evaluate whether or not ER and PR are expressed in formalin-fixed, paraffin-embedded tissue samples of ACC, and to consider the issue of whether positive results suggest that endogenous estrogen or progesterone might influence the course of development of some cases of ACC.

A second goal of this study is to access whether p53, the most common multifunctional gene aberration identified in human malignancy presents with any frequency in ACC of salivary glands. Since little is known about the specific role of p53 tumor suppressor gene mutation and altered p53 protein expression as it relates to the pathology and ultimate biologic behavior of ACC, it was deemed pertinent to evaluate whether p53 is in fact expressed to any degree in ACC.

Materials and Methods

Forty-seven ACCs were retrieved from the Surgical Pathology archives of the University of Colorado, Schools of Medicine and Dentistry, Western States Pathology Laboratory and Oral Pathology Associates, Inc., and the Section of Oral Pathology at the New York Hospital of Queens. Institutional review board approval was obtained to examine archival specimens.

Tumor Subtyping

Adenoid cystic carcinomas rarely occur in any pure histologic form, but rather most often as an admixture of cribriform, tubular or solid types. For this study we developed a method of classifying these three grades of tumors that required that at least 70% of any tumor have either a predominant cribriform, tubular or solid pattern. Thus if 70% of the tumor showed any one pattern it was sub classified into that group regardless of any other histologic pattern observed. Hematoxylin and Eosin (H & E) stained slides were reviewed by two pathologists to confirm diagnoses.

Estrogen and Progesterone Localization

Formalin-fixed paraffin-embedded tissues were analyzed using an integrated histopathologic immunohistochemical approach. Four micron thick tissue sections were immunohistochemically stained for ER and PR using a method developed in the laboratory for Lymphoproliferative Diseases in the Department of Pathology, University of Colorado Health Sciences Center. Tissues were deparaffinized in a 37°C warm up slide chamber, rinsed, and incubated, using 3% H_2O_2 for 4 min. Following rinsing, one drop of antibody for both anti-progesterone and anti-estrogen, (Ventana) was applied and the sample was incubated for 8 min. Following a rinse with amplifier B, the tissue was incubated again for 8 min. A DAB kit (Ventana) was then used with appropriate incubation and the tissue was ultimately counter stained with light hemoatoxylin. Immunoreactivity was scored using a 0 to +3 scale in which staining was, negative (0), spotty (+1), weakly positive (+2) or strongly positive (+3).

p53 Localization

Paraffin sections were deparaffinized and hydrated through water. Endogenous peroxidase was blocked in 0.02% hydrogen peroxide in water. Sections were then microwaved for 15 min in citrate buffer. After rinsing in PBS, sections were treated in 10% normal goat serum at 30°C for 20 min. Excess serum was tapped off and sections were then incubated in primary monoclonal p53 antibody, 1 μg/ml (Oncogene, cat#OP43) overnight at 4°C. Primary antibody was rinsed from the sections and antigen activity was visualized using Biogenix strAviGen Multilink kit (cat#LP000-ul) with DAB chromagen. Positive and negative controls were performed for each experiment.

Results

We were able to demonstrate ER overexpression in 8 of 47 tumors, PR overexpression in 4 of 47 tumors and p53 overexpression in 26 of 47 tumors. All lesions were compared with similarly fixed and prepared normal salivary gland tissue (Table 1).

Patients ranged in age from 24 to 99 years of age and 11 of the 47 patients were males. Patients selected were screened for evidence of prior cancer or endocrine disease.

Table 1 Expression of ER, PR and p53 in ACC

Case no.	Histologic subtype	p53	ER	PR	Age	Sex	Site
1	Cribriform	+1	+2	Neg	85	F	Maxilla
2	Tubular	Neg	+2	+1	82	F	Palate
3	Tubular	+2	Neg	Neg	57	F	Maxillary tuberosity
4	Cribriform	Neg	Neg	Neg	70	F	Ant. Tonsillar Pillar
5	Solid	+1	Neg	Neg	65	F	Palate
6	Cribriform	Neg	Neg	Neg	65	F	Upper lip
7*	Solid	+1	Neg	Neg	99	F	Mandible
8	Cribriform	+1	Neg	Neg	86	M	Palate
9	Cribriform	Neg	Neg	+1	82	F	Maxillary tuberosity
10	Cribriform	+3	Neg	Neg	46	F	Palate
11	Solid	+2	+2	Neg	70	M	Palate
12	Solid	Neg	Neb	Neg	68	F	Palate
13*	Cribriform	Neg	Neg	Neg	51	F	Submand gland
14	Cribriform	Neg	Neg	Neg	45	F	Palate
15	Cribriform	Neg	Neg	Neg	46	M	Submand. gland
16	Cribriform	+2	+2	+1	55	F	Mandibular vestibule
17	Cribriform	+2	Neg	+1	72	F	Palate
18	Cribriform	+1	Neg	Neg	42	F	Palate
19*	Cribriform	+1	Neg	Neg	24	F	Palate
20	Cribriform	+1	Neg	Neg	72	M	Floor of mouth
21	Cribriform	+3	Neg	Neg	61	F	Buccal mucosa
22	Cribriform	+1	Neg	Neg	66	F	Mandible
23	Tubular	+1	Neg	Neg	85	F	Mandible
24	Cribriform	Neg	Neg	Neg	45	F	Floor of mouth
25	Cribriform	+2	+1	Neg	71	M	Floor of mouth
26	Cribriform	Neg	Neg	Neg	37	M	Maxilla
27	Cribriform	+2	Neg	Neg	42	F	Palate
28	Solid	Neg	Neg	Neg	66	F	Maxilla
29	Cribriform	Neg	Neg	Neg	74	F	Tongue
30	Cribriform	Neg	Neg	Neg	50	F	Maxillary vestibule
31	Cribriform	+1	Neg	Neg	33	M	Hard palate
32	Cribriform	+1	Neg	Neg	44	F	Maxilla
33	Cribriform	+1	Neg	Neg	40	F	Hard palate
34*	Solid	Neg	Neg	Neg	50	M	Floor of mouth
35	Tubular	+1	Neg	Neg	30	F	Palate
36	Cribriform	+1	Neg	Neg	41	F	Hard palate
37	Cribriform	+2	Neg	Neg	62	F	Upper lip
38	Cribriform	Neg	+2	Neg	67	F	Floor of mouth
39	Cribriform	Neg	Neg	Neg	35	F	Floor of mouth
40	Cribriform	+2	Neg	Neg	49	M	Maxilla
41	Tubular	Neg	Neg	Neg	31	M	Hard palate
42	Cribriform	+1	Neg	Neg	50	F	Tongue
43	Solid	Neg	Neg	Neg	77	F	Left maxilla
44	Cribriform	Neg	Neg	Neg	39	F	Rt. Buccal mucosa
45	Cribriform	Neg	Neg	Neg	92	F	Rt. Buccal mucosa
46	Cribriform	Neg	+1	Neg	60	M	Palate
47	Cribriform	+1	+1	Neg	52	F	Palate

*Recurrent tumors (7, 13, 19, 34)

The menopausal status of the female patients was not correlated in the study. Thirty-five of the cases examined were sub-classified histologically as having a cribriform pattern. Five cases demonstrated a tubular pattern, and seven cases showed a solid pattern.

Histopathology and Immunopathology

The intensity of the staining in the eight ACCs that demonstrated ER overexpression were graded as +2 in five cases while three cases were graded as +1, with no cases showing greater than half of the cells staining using our semi quantitative intensity scale (Fig. 1). No cases demonstrated +3 staining. Six cases were identified as cribriform histologic variants, one case was tubular and one case was solid.

Four of 47 tumors demonstrated PR overexpression. None of the tumors demonstrating PR overexpression showed greater than spotty +1 immunoreactivity (Fig. 2). Interestingly two cases, case numbers 2 and 16 also showed ER overexpression.

Twenty-six of 47 cases showed p53 overexpression. The intensity of the staining in the 26 cases that were p53 positive ranged from 0 to +3, with no cases showing greater than 60% of cells staining using our semi-quantitative intensity scale. p53 immunopositive cells tended to be evenly distributed within the tumor islands (Figs. 3 and 4).

ER, PR, and p53 staining was negative for all control tissue. No histologic subtypes of ACC, either tubular, cribriform or solid demonstrated overexpression of either ER, PR, or p53 in a manner that would suggest that the markers could be linked to biologic behavior of a specific histologic subtype. p53 immunopositivity was found in 20 cribriform, three solid, and three tubular histologic subtypes. One recurrent tumor was p53 immunopositive, but ER and PR immunonegative. Only one case of 47, case 16, was immunopositive for ER, PR, and p53; this case was a cribriform histologic variant.

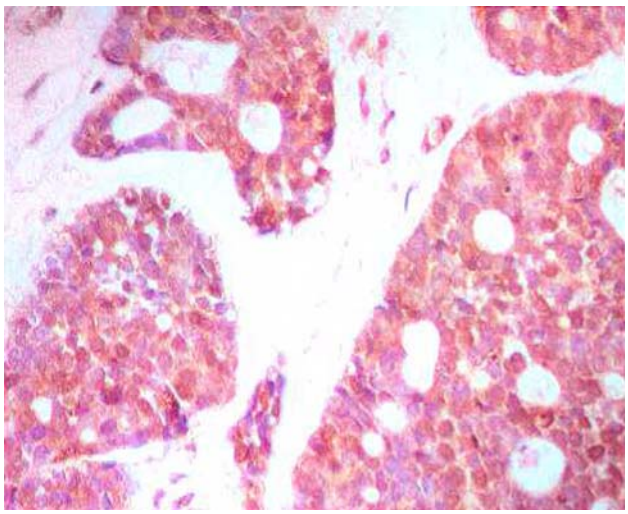

Fig. 1 Minor salivary gland adenoid cystic carcinoma, cribriform histological subtype showing (+2) immunostaining for ER

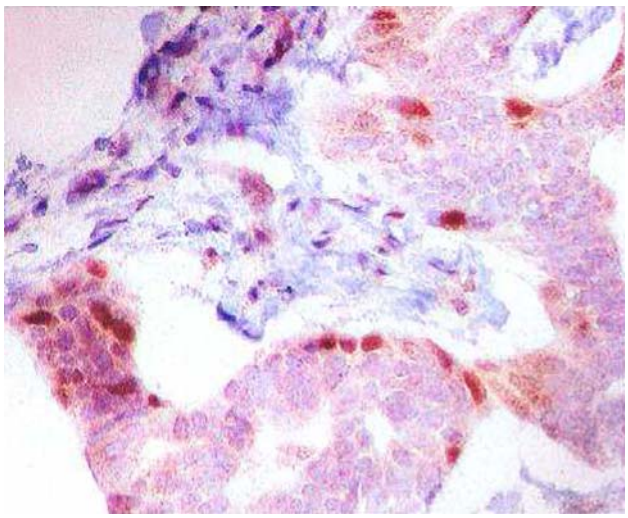

Fig. 2 Minor salivary gland adenoid cystic carcinoma, tubular histological subtype showing (1+) immunostaining for PR. Magnification, 200×

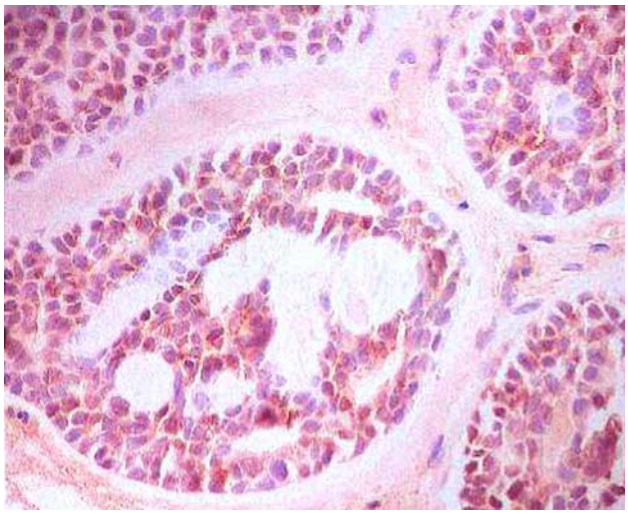

Fig. 3 Minor salivary gland adenoid cystic carcinoma, cribriform histological subtype, and demonstrating (+2) immunostaining for p53. Magnification, 200×

Discussion

The current study allowed direct visualization of ER and PR immunopositive cells in ACC. Estrogen receptor and PR expression are well established in breast carcinoma and their presence has been shown to confer a more favorable

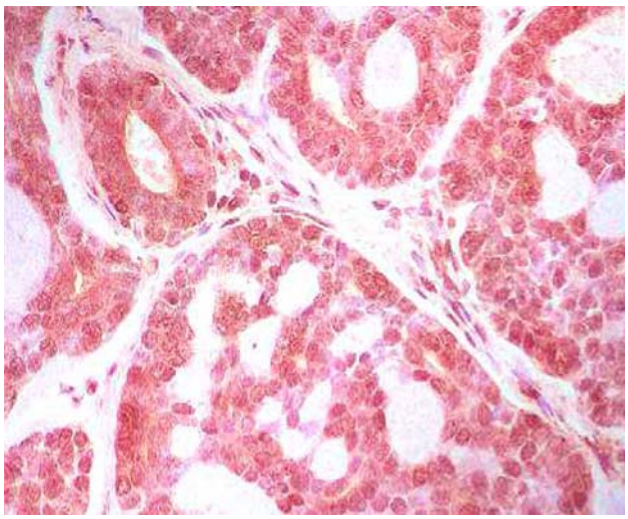

Fig. 4 Minor salivary gland adenoid cystic carcinoma, cribriform histological subtype, and demonstrating (+3) immunostaining for p53. Magnification, 200×

prognosis for the patients. This more favorable prognosis is related to the fact that such tumors are more susceptible to hormonal manipulation therapy [11–13]. Some reports suggest that the presence of ER and PR in salivary glands, and salivary gland ACC may offer a similar favorable prognosis [5,14].

Shick et al. [5] evaluated 12 ACCs of salivary gland origin and found that ER were not detectable. These same investigators, however, demonstrated a significantly higher PR level in ACC than in matched normal salivary gland controls. Miller et al. [15] evaluated a group of five ACCs using an immunocytochemical assay for identification of human ER in formalin-fixed tissue. These authors were unable to demonstrate ER in any of the five cases of ACC using a 4-point grading scale. Dori et al. [16] did not detect ER in 27 samples and only two cases detected PR in his study.

Numerous factors, including patient age, tumor histopathology, gender, anatomic location, and immunohistochemical technique may account for these reported differences in ER expression. Interpretation of staining due to a less than strict evaluation criteria for tumors may also account for these differences. The studies of Ozono et al. [14], and Shick et al. [5], as well as the current study support the fact that progesterone can be identified, although rarely, in ACC and estrogen can be ever more readily identified.

In the current study, we were able to identify not only estrogen and PR in ACC, but overexpression of p53 as well. We found no gender or age predilection to be associated with the presence of any of the three markers. Our findings are in concert with those of Dimery et al. [4] who found ER expression in 14 samples (75%) of ACC studied.

It is interesting that in the study of Shick et al. [5] 50% of the tumors stained positively for PR. This finding is similar to those reported for breast carcinomas [11]. Ozono et al. [14] reported that 80% of five salivary gland ACCs that they studied stained positively for PR. Their study, however, did not use a grading scale equivalent to the one used in the current study, therefore the evaluative parameters are not fully comparable.

The presence of ER in ACC of the breast is rather widely accepted as an indicator of potential hormone responsiveness. There have been numerous studies to support the fact that this finding may offer inroads into understanding the efficacy of endocrine treatment for ACC of the breast. The prognostic significance of PR assessment and its measurement in breast carcinomas has not been definitely proven to be of significant value. Ozono et al. [14], in their study of six cases of primary ACC in the submandibular glands, parotid glands, and minor salivary glands, suggest that the presence of a PR may be a good indication of tumor hormone dependence. Thus ACC of salivary glands, like ACC of the breast appears to have the potential of responding to hormone stimulation because of the presence of PR. The presence of ERs in ACC of salivary glands may be associated with the presence of ER in tumor cells and tumor regression may well follow hormone therapy. However to support this concept, further studies need to be pursued in this arena.

There have been few definitive studies that compare the staining of breast ACC with ACC of salivary glands, although when one compares the staining between ACC of the breast, as reported by Trendell-Smith et al. [17], with ACC of salivary glands, the staining of cells is relatively equivalent. It is possible that the marked amount of reduplicated basement membrane substance that is common to ACC of the salivary glands, a less frequent finding in ACC of the breast, may result in a different staining pattern in the two anatomic sights, but to date definitive comparative studies other than those of Trendell-Smith et al. [17], have not been reported.

The clinical laboratory scoring of ER/PR receptors for breast tumors used in many laboratories and typically designated as: negative, low positive and positive was not used in this study. Instead, the scoring system used here was a modification of the system initially developed by Shick et al. [5] and modified by Ozona et al. [14] for evaluating ACC of minor salivary glands, for research rather than clinical laboratory purposes.

It may be wise in future studies for investigators to use a clinical scoring system similar to the one used for breast cancer in the evaluation of salivary gland ACCs since immunoreactivity is an important evaluative factor in ACC of the breast and very probably for salivary gland as well,

but such immunoreactivity was not evaluated in the current study.

Kiyoshima et al. [18] demonstrated a 17.6% expression for p53 (3 of 17 ACC cases). All of these samples were recurrent or metastatic ACC. In our study, p53 expression was found in 55% of cases (26 of 47 primary ACC tumors). None of the previous studies evaluating ACC for p53 examined the concomitant overexpression of either ER or PR. It is thus considered important to further investigate the role of p53 overexpression in ACC to more adequately determine if this mutation is significant in early, mid, or late stage events as they relate to the evolution of the tumor, and whether p53 expression in ACC might be ER or PR dependent. Recently, active estrogen signaling pathways attenuated p53 apoptotic responses through increased MDM2 transcription, a polymorphism of the p53 tumor suppressor pathway, leading to tumor formation [10].

Some authors have suggested that PR expression may be a better marker of tumor hormone dependence than ER expression [5], but in the current study PR positive staining was spotty in all four cases that were positive. In addition, ER expression was found in 17% of cases while PR expression was detected in only 8.5% of cases. Only one case, a cribriform variant, demonstrated expression for all three markers. None of the histologic subtypes of ACC: tubular, solid, or cribriform routinely demonstrated overexpression of ER, PR or p53, therefore we were unable to demonstrate whether any of these markers can be related to histologic subtype, or tumor aggression.

The bulk of our studies involved minor salivary gland sites, with only two of 47 cases coming from major salivary glands. In evaluating these 47 ACCs, we found no differences in immunohistochemical staining between major and minor salivary gland sites, but it may be of interest and importance to compare the staining incidence, intensity and quality between major and minor salivary gland ACCs, which we plan to do in future studies.

Conclusions

In the current study, immunohistochemical staining methods enabled us to identify expression of p53, ER and PR in salivary gland ACCs. We believe that further studies are indicated to help further define the mechanisms of action and the possible uses of hormonal therapy in the management of ACC as they relate to ER, PR and p53 status.

Acknowledgements This work was supported by a grant from the National Organization for Rare Disorders, Inc.

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